AHNAK controls 53BP1-mediated p53 response by restraining 53BP1 oligomerization and phase separation

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Abstract: p53-binding protein 1 (53BP1) regulates both the DNA damage response and p53 signaling. Although 53BP1's function is well established in DNA double-strand break repair, how its role in p53 signaling is modulated remains poorly understood. Here, we identify the scaffolding protein AHNAK as a G1 phase-enriched interactor of 53BP1. We demonstrate that AHNAK binds to the 53BP1 oligomerization domain and controls its multimerization potential. Loss of AHNAK results in hyper-accumulation of 53BP1 on chromatin and enhanced phase separation, culminating in an elevated p53 response, compromising cell survival in cancer cells but leading to senescence in non-transformed cells. Cancer transcriptome analyses indicate that AHNAK-53BP1 cooperation contributes to the suppression of p53 target gene networks in tumors and that loss of AHNAK sensitizes cells to combinatorial cancer treatments. These findings highlight AHNAK as a rheostat of 53BP1 function, which surveys cell proliferation by preventing an excessive p53 response.

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AHNAK controls 53BP1-mediated p53 response by restraining 53BP1 oligomerization and phase separation

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SUMMARY

p53-binding protein 1 (53BP1) regulates both the DNA damage response and p53 signaling. Although 53BP1's function is well established in DNA double-strand break repair, how its role in p53 signaling is modulated remains poorly understood. Here, we identify the scaffolding protein AHNAK as a G1 phase-enriched interactor of 53BP1. We demonstrate that AHNAK binds to the 53BP1 oligomerization domain and controls its multimerization potential. Loss of AHNAK results in hyper-accumulation of 53BP1 on chromatin and enhanced phase separation, culminating in an elevated p53 response, compromising cell survival in cancer cells but leading to senescence in non-transformed cells. Cancer transcriptome analyses indicate that AHNAK-53BP1 cooperation contributes to the suppression of p53 target gene networks in tumors and that loss of AHNAK sensitizes cells to combinatorial cancer treatments. These findings highlight AHNAK as a rheostat of 53BP1 function, which surveys cell proliferation by preventing an excessive p53 response.

INTRODUCTION

The tumor suppressor protein p53 plays a pivotal role in triggering multiple signaling pathways in response to a wide range of cellular stresses. Mechanistically, upon sensing stress, p53 rewires pan-genomic transcriptional programs, which includes the induction of cell-cycle arrest (e.g., CDKN1A/p21, 14-3-3σ), pro-apoptotic (e.g., BAX, TP53I3, PUMA,), and senescence (e.g., FAS, PDID) genes. In response to DNA damage, p53 activation is triggered by the ATM/ATR kinases, the apical responders to DNA double-stranded breaks (DSBs), and replication stress, respectively. ATM/ATR-mediated phosphorylation of p53 and of its inhibitor MDM2 initiate a converging mechanism, leading to the stabilization of p53 (Meek, 2004). The primary target of activated p53 is a multifunctional protein, p21, which attenuates cyclin/CDK activity and instigates G1/S cell-cycle arrest. p21 is also known to activate genes involved in senescence (Meek, 2004; Mirzayans et al., 2012). In a p53-deficient background, cells have an impaired G1/S checkpoint, resulting in the propagation of structural aneuploidies and early onset of carcinogenesis (Soto et al., 2017). Other studies (Abbas and Dutta, 2009) have shown that the strength of the p53 response dictates the balance between cell division and cell-cycle arrest.

p53-binding protein 1 (53BP1) was identified as an interactor of p53 (Iwabuchi et al., 1994) and is positioned at the crossroads of DSB repair and p53 signaling. It harbors key structural elements, including 28 N-terminal Ser/Thr-Gln (S/T-Q) sites, a central minimal focus-forming region (MFFR) composed of an oligomerization domain (OD), a Gly- and Arg-rich (GAR) motif, a tandem Tudor domain, a ubiquitylation-dependent recruitment (UDR) motif, and a C-terminal BRCT domain. Whereas both the OD and BRCT domains are critical for p53 activation, the BRCT domain is dispensable for DNA repair (Mirman and de Lange, 2020). 53BP1 is stabilized on chromatin by UDR motif-mediated binding to H2AUb15, and via binding of its tandem Tudor domain to mono- and dimethylated H4K20 (Panier and Boulton, 2014). Domains flanking the Tudor domain (i.e., OD and the LC8 domain) drive multimerization of 53BP1 and promote DNA damage-dependent 53BP1 recruitment to the chromatin (Becker et al., 2018; Sundaravinayagam et al., 2019; Ward et al., 2006; Zgheib et al., 2009).